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Direct transfer of a *Mycoplasma mycoides* genome to yeast is enhanced by removal of the mycoides glycerol uptake factor gene glpF

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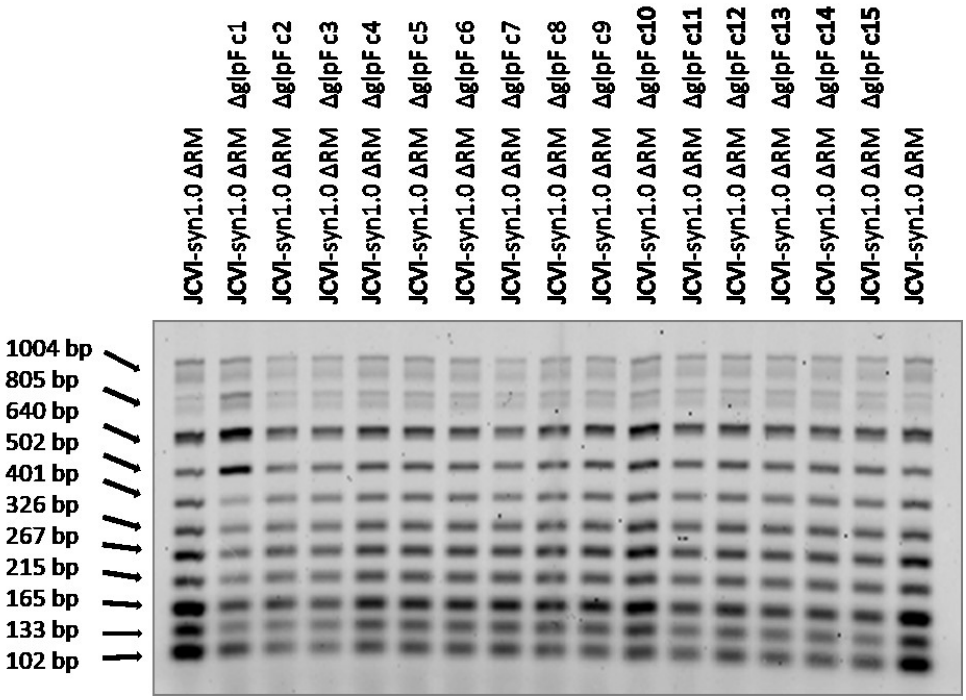

Supplementary Figure S1. Characterization of genomes transferred to yeast. Multiplex screen was performed for 15 yeast colonies that came from the experiment where JCVI-syn1.0 ΔRM ΔglpF was used as donor strain. As a control, genomic DNA from JCVI-syn1.0 ΔRM was used (lane 1 and 17). Primers used for this experiment were described in our pervious publication[1]

Supplementary Table S1. Primers used to generate deletion cassettes and for confirmation of insertion of deletion cassettes. Underline is the sequenced that binds to the template URA3 gene.

Primers to generated glpO deletion cassette	
MmycglpOKOF	GTTAAGATGTATTTTTTACTATCTGTCATAGTTATTCTCCTTATAC<u>GTTGCAGGCCATGC</u>
MmycglpOKOR	TCAAGTAATTCTTATAAAATTTTGAATTTTTTAAAAAAGAATATTC<u>GATGAAACGAGAGA</u>
Primers to confirm correct integration of deletion cassette at 5' and 3' ends for glpO	
MycglpOcheckF1	TGACTTGGGTCAATTCCAGA
MycglpOcheckR1	GCTTCCATTCAGGTCGAGGT
MycglpOcheckF2	GGCATTGACCCTGAGTGATT
MycglpOcheckR2	GAAAGGAGAAAACTAAATAACTGTTCA
Primers to generated glpK deletion cassette	
MmycglpkKOF	AACATGTTATCCTTTCATTATTATTTAATTTTTTAAAAATGTAAAC<u>GTTGCAGGCCATGC</u>
MmycglpkKOR	AAGAAGAAGCTATTCTTCCATGAAAATAGTATAAGGAGAATAACT<u>CGATGAAACGAGAGA</u>
Primers to confirm correct integration of deletion cassette at 5' and 3' ends for glpK	
MycglpKcheckF1	CAGCTGATATTCCAGCAGCA
MycglpKcheckR1	GCCTACAATCCATGCCAAC
MycglpKcheckF2	CCATTATGTTCCGGATCTGC
MycglpKcheckR2	ACCAACCATTCACGGAAAAG
Primers to generated glpF deletion cassette	
MmycglfFKOF	AATTGATGAGCTTTTCTTTTGCTTAATTTATTAAGTTATAAAATTC<u>GTTGCAGGCCATGC</u>
MmycglFKOR	AATAATTTACATTTTTAAAAAATTAAATAATAATGAAAGGATAACC<u>GATGAAACGAGAGA</u>
Primers to confirm correct integration of deletion cassette at 5' and 3' ends for glpF	
MmycglFchekF1	CCAAGCGGTAGAAAAATTGA
MmycglFchekR1	GTCCCTGATGGTCGTCATCT
MmycglFchekF2	CCATTATGTTCCGGATCTGC
MmycglFchekR2	GCTGCATTTATGGCAGGATT

References

1. Karas, B. J. *et al.* Direct transfer of whole genomes from bacteria to yeast. *Nat. Methods* **10,** 410–2 (2013).